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Research Article

Antinociceptive Efficacy of Flavonoids Rich Latex Extract from *Pergularia daemia* involving L-arg/nitric oxide/cGMP/K^+ ATP channels Pathway in Experimental Animals

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ABSTRACT

Pergularia daemia is a hispid perennial herb, its latex used in traditional ayurvedic and siddha medicine for migraine, head ache, muscle sprain and swelling sores, boils. Despite of its pain relieving potential, the mechanisms of these effects have yet to be elucidated. The methanolic flavonoids rich *Pergularia daemia* latex (PDL) extract subjected into particular peripheral and central antinociceptive effects in laboratory animals. The present study was to determine the involvement of nitric oxide (NO)/cyclic guanosine monophosphate (cGMP)/ potassium (K^+) channels pathway in the peripheral antinociception induced by flavonoids rich PDL. It was executed systemically for (50, 100 and 200 mg/kg p.o.) to generate dose dependent antinociception when evaluated using acetic acid induced abdominal writhing and formalin test with measured mean IC_{50} value of 169.9 mg/kg, 136.6 mg/kg and 174.8 mg/kg respectively. PDL at similar doses showed significant dose dependent inhibition of neurogenic pain induced by capsaicin (1.6 g/paw/ IC_{50} = 194.9 mg/kg) and glutamate (10 µmol/paw/ IC_{50} = 204.6 mg/kg) which elicits the participation of vanilloid (TRPV) receptors and glutamatergic system. Moreover, PDL displayed reversed antinociception with L-arginine (a NO precursor) (100 mg/kg, s.c.), implying the involvement of L-arginine/nitric oxide pathway besides methylene blue (10 mg/kg, s.c.) remarkably increased the antinociception and an ATP-sensitive K^+ channel antagonist glibenclamide (10 mg/kg, i.p.) reversed antinociceptive activity when administered systemically induced by PDL. Conjointly, the results suggested that PDL induced antinociceptive activity was possibly related to inactivate TRPV receptor, glutamatergic system along with the activation of NO/cGMP/ATP sensitive K^+ channel pathway.

Keywords: Antinociceptive, *Pergularia daemia*, L-arginine, Vanilloid receptors, Glutamatergic system, NO/cGMP/ATP K^+ channel.

INTRODUCTION

The subfamily Asclepiadoideae (Milkweed) belongs to family Apocynaceae is amid the most plenteous plants in tropical and subtropical regions [1], which is flourishing naturally as perennial herbs are mostly abundant in temperate zones besides that some are distributed in tropical dry environments as well [2]. *Pergularia daemia* (Forsk.) chiov is a hispid perennial herb, grows along the roadsides of India. Traditionally it has been used for laxative, antipyretic, amenorrhea, anthelmintic, expectorant and infantile diarrhoea [3-5]. Interestingly, its latex is used for oedema, Rheumatism, Kidney pains, boils and sore eyes [6-8], in particular for migraine, head and ear ache. According to antecedent studies, diverse groups of secondary metabolites, such as alkaloids, cardenolides, flavonoids, saponins, triterpenoids, and steroidal compounds are effectively present [9-11] and recent pharmacological studies revealed the potential applications of different parts of *P. Daemia* in anti-inflammatory, analgesic and antipyretic [12], Antidepressant [13], hepatoprotective [14], anti-diabetic [15] and anti- fertility [16]. Despite of numerous studies on *Pergularia daemia*, the role of latex alone is yet not characterized even it has remarkable ethno pharmacological background such as antimigraine [17-18]. Moreover, the mechanism of action behind the PDL exerts its antinociceptive potential remains to be elucidated. From this study PDL possessed both central and peripheral antinociceptive activity when examined using chemical models. In particular, the PDL did not develop any toxic and sedative effects. Many studies have exhibited that NO/cGMP and K^+ channels, modulate pain perception which transduces pain signals at peripheral and central levels and played a crucial role in the antinociceptive activity of analgesic drugs (opioids and NSAIDs). Besides, the K^+ channels opened by the activation of NO/cGMP pathway in the peripheral and central nervous system, which contributes to antinociceptive mechanisms [19]. At very first time, this study was aimed to figure out the antinociceptive effects of PDL and also examines its mechanism, by means of pharmacological approaches, the role of the NO/cGMP/ATP sensitive K^+ channel pathway in its analgesic effects. Thereby, the current study provides further insights into the mechanism of antinociception induced by PDL. Certainly, it leads us

to understand about the mode of action of an analgesic agent for its better clinical application.

Plant latex Collection

The fresh latex from leaves was secured from in and around areas of Thiruvathavur village, Melur Taluk, Madurai district, Tamilnadu, India. The taxonomic identification and authentication was done by Dr. C. Murugan, Scientist 'D'& Head, Botanical survey of India, Coimbatore, and Tamilnadu state. A voucher specimen (BSI/SRC/5/23/2018/TECH/1674) was deposited at the Herbarium of the Department of Natural products chemistry, School of chemistry, Madurai Kamaraj University, Madurai. The latex was kept in freezer at 4 ºC until use.

Preparation of Flavonoids rich extract

Based on the methodology followed by Mabry et al [20] with minor modifications, the fresh latex 500 mL was homogenized in a homogenizer and dried in oven at 36 ºC to obtain 30% dry powder. About 100 g of dried latex was extracted with cold 20% methanol for 48 h. The solvent was evaporated under in vacuum which yielded about 15 g of sticky syrup which was dissolved in 100 mL of hot methanol. This solution mixed with celite and then activated charcoal and filtered. The remaining charcoal-flavonoid material was eluted with 1L of boiling solution of phenol: water (7:93). Then, the filtrate was evaporated under vacuum (about 80 ºC and 12 mm pressure) and yielded 5.2 g of flavonoid rich residue.

GC-MS analysis of PDL

GC–MS analysis of PDL was performed using Agilent 7820A (Agilent Technologies) coupled with MSD quadrupole detector 5977E (Agilent Technologies). The separation of analytes by gas chromatography was carried out using the Hewlett Packard HP-5MS (ultra inert) silica capillary column (30 m × 250 μm × 0.25 μm). For GC–MS detection, an electron ionization system with ionizing energy of 70 eV was used. Helium gas (99.999%) was used as the carrier gas at constant flow rate 1 ml/min and an injection volume of 1L was employed (split ratio of 1:10); injector temperature 250ºC; ion-source temperature 280ºC. Total GC running time was 23 min. The relative % amount of each component was calculated by comparing its average peak area to the total areas. For the identification of compounds, interpretation on mass spectrum GC–MS was conducted using the database of National Institute Standard and technology (NIST) having more than 62,000 patterns.

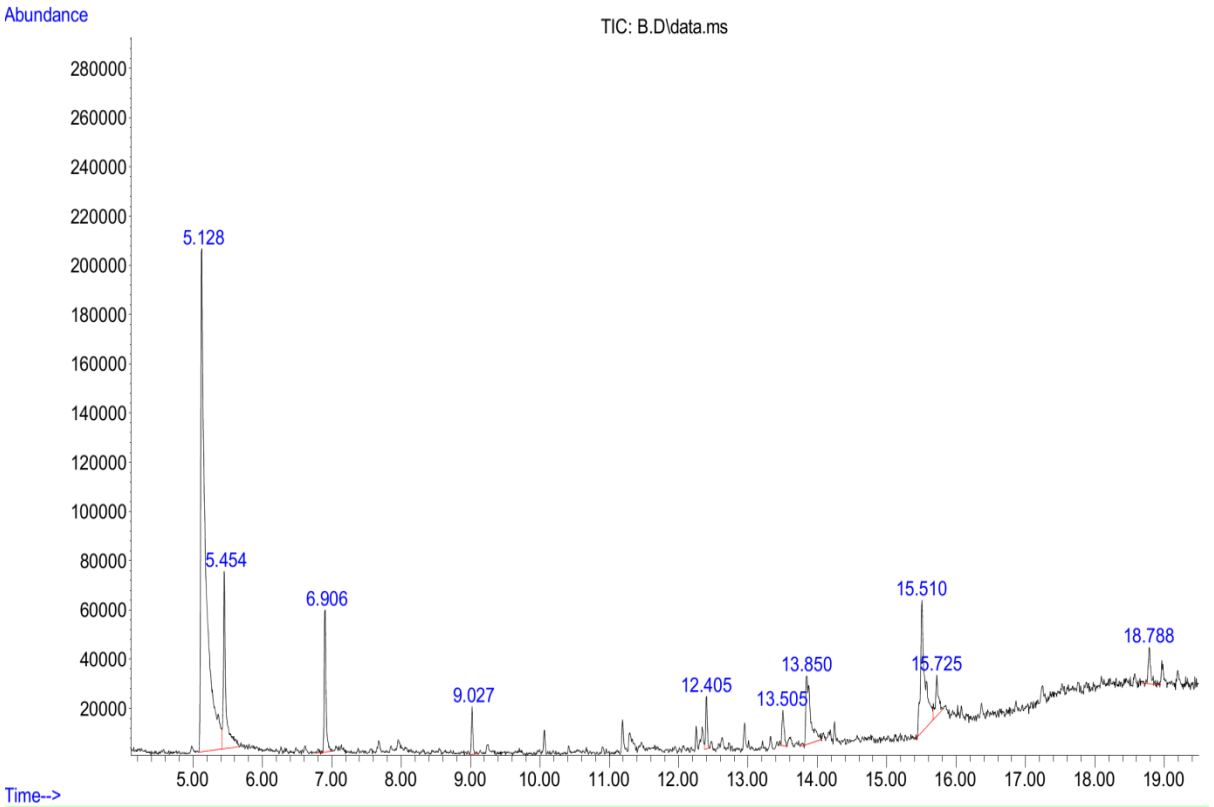

Fig. 1. GC-MS chromatogram of flavonoid rich PDL crude

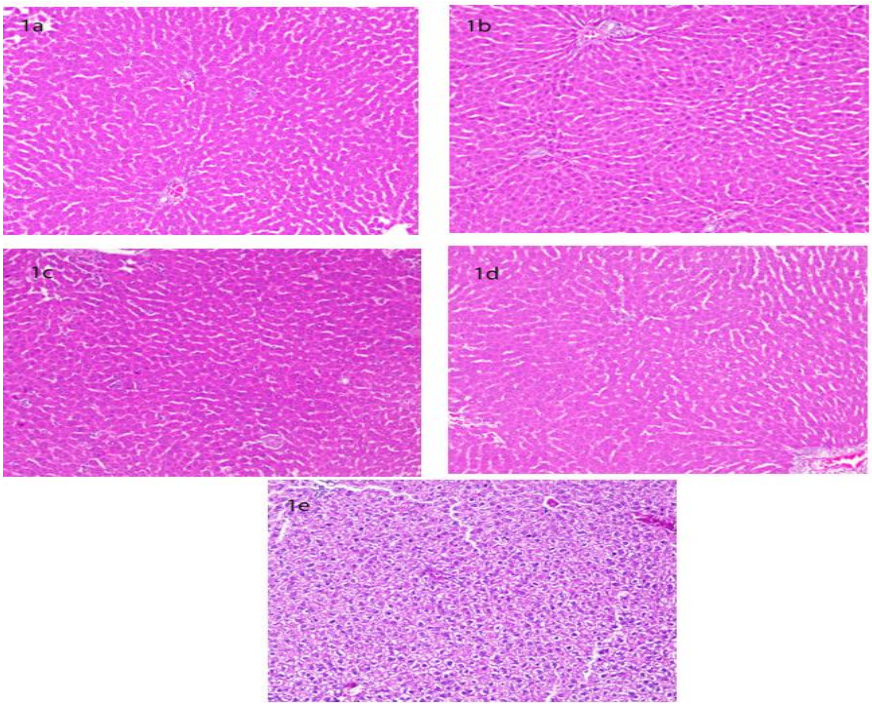

Fig. 2. Histopathological liver images of PDL ingested mice. Three different doses 30, 300, 2000 mg/kg (1a-1c), Control (1d), Toxic control (1e)

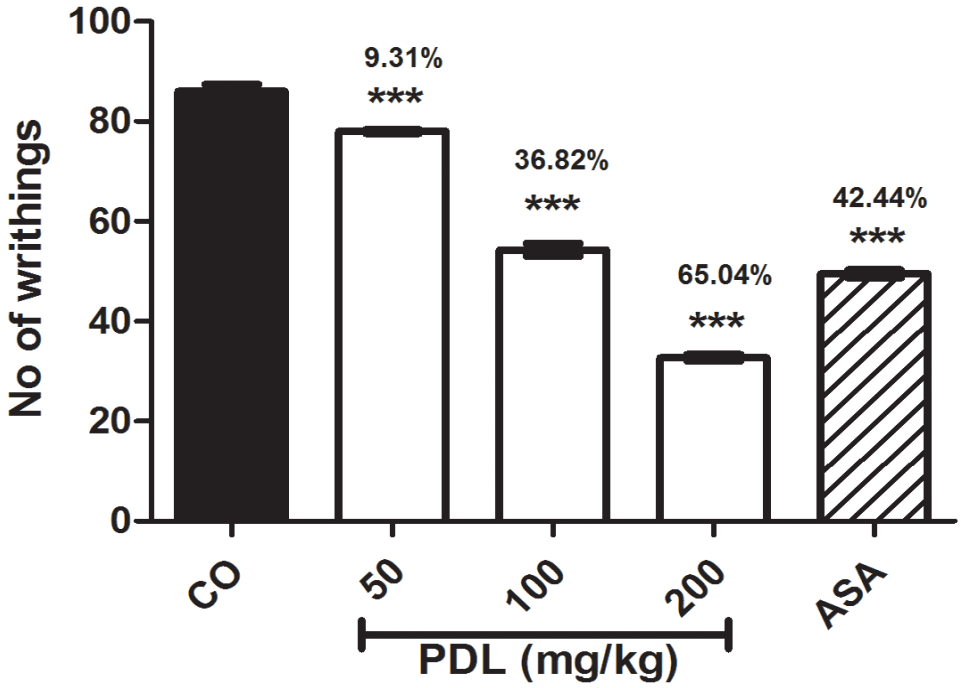

Fig. 3. Effects of PDL at the dose 50-200 mg/kg on Acetic acid- induced writhing in mice. CO: Control group. Values are mean ± SEM (n=6). ***P<0.001, asterisks denote the significance levels compared with the control group (one-way ANOVA, followed by Dunnett's post hoc test)

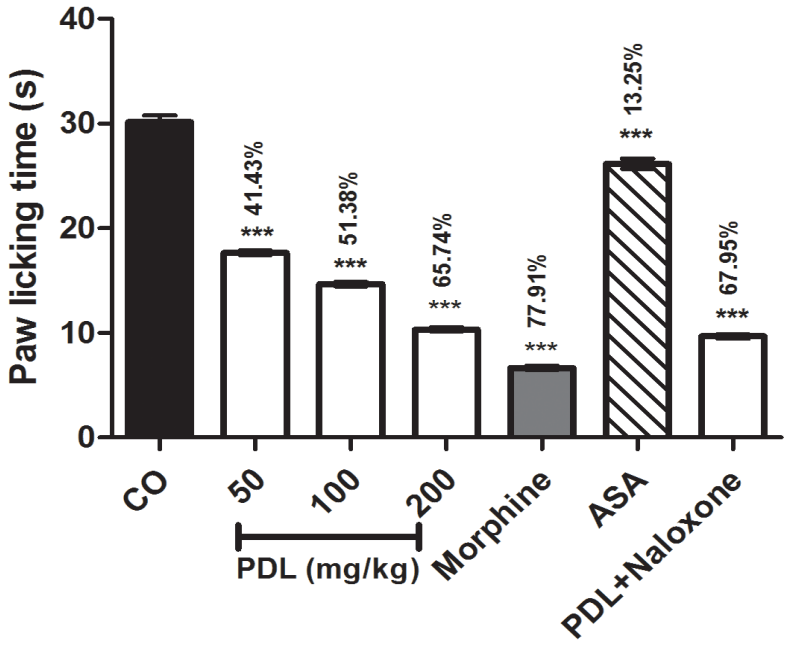

Fig. 4a. Formalin induced antinociceptive activities of PDL at the dose 50-200 mg/kg in First phase. CO: Control group. Values are mean ± SEM (n=6). ***P<0.001, asterisks denote the significance levels compared with the control group (one-way ANOVA, followed by Dunnett's post hoc test)

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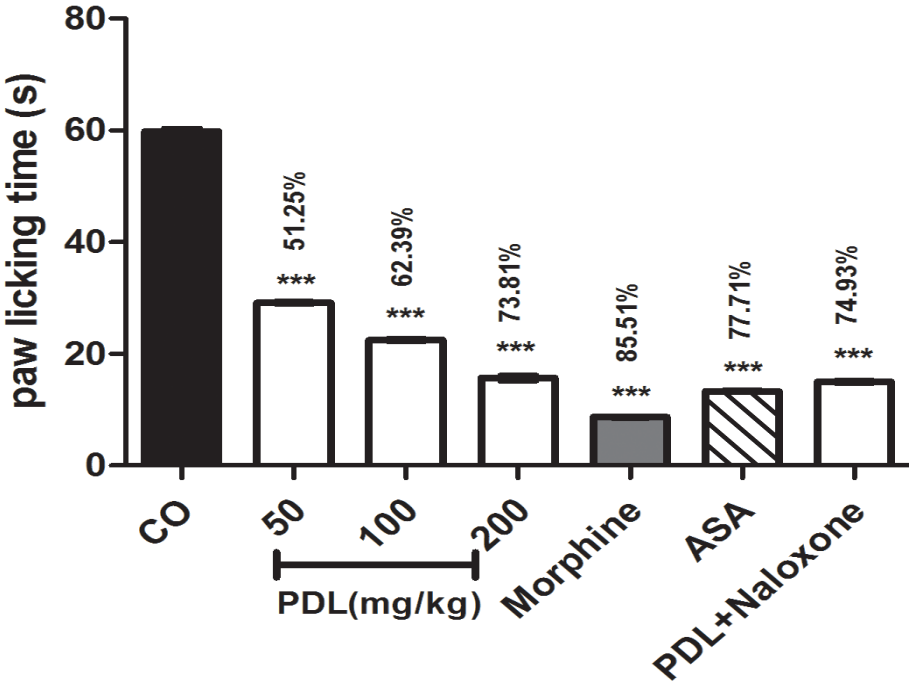

Fig. 4b. Formalin induced antinociceptive activities of PDL at the dose 50-200 mg/kg in Second phase. CO: control group. Values are mean ± SEM (n=6). ***P<0.001, asterisks denote the significance levels compared with the control group (one-way ANOVA, followed by Dunnett's post hoc test)

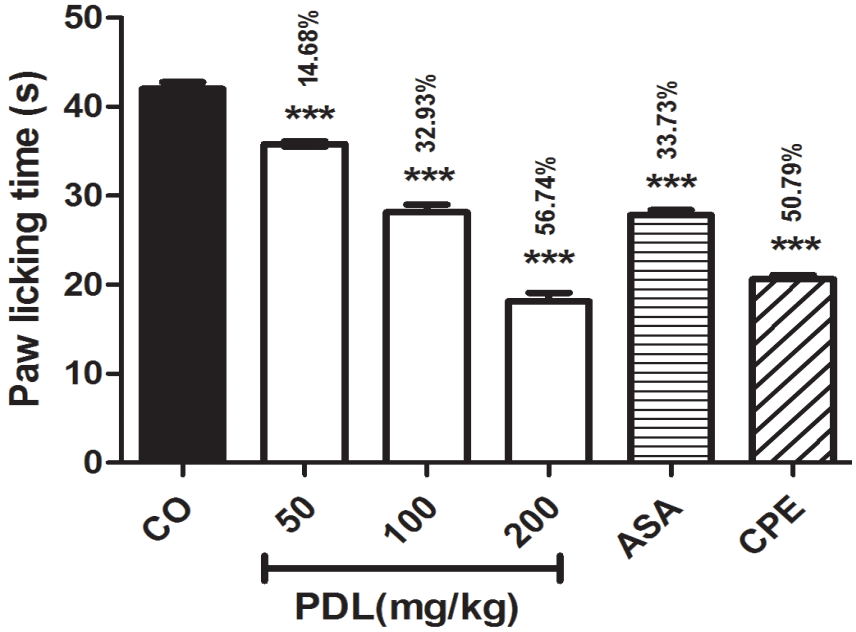

Fig. 5. Effects of PDL at the dose 50-200 mg/kg on Capsaicin- induced nociception. CO: control group. Capsazepine (CPE) and Aspirin (ASA) are used as the positive control. Values are mean ± SEM (n=6). The asterisks denote the significance levels compared with the control group (one-way ANOVA, followed by Dunnett's post hoc test): ***P<0.001.

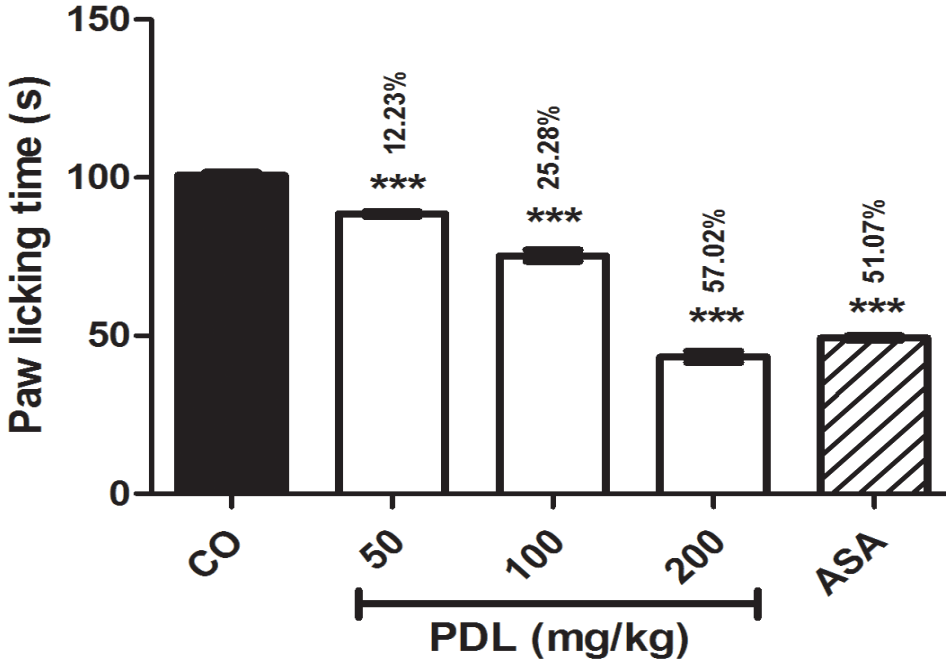

Fig. 6. Effects of PDL at the dose 50-200 mg/kg against Glutamate- induced nociception. CO: Control group. Aspirin (ASA) is used as the positive control. Values are mean ± SEM (n=6). The asterisks denote the significance levels compared with the control group (one-way ANOVA, followed by Dunnett's post hoc test): ***P<0.001.

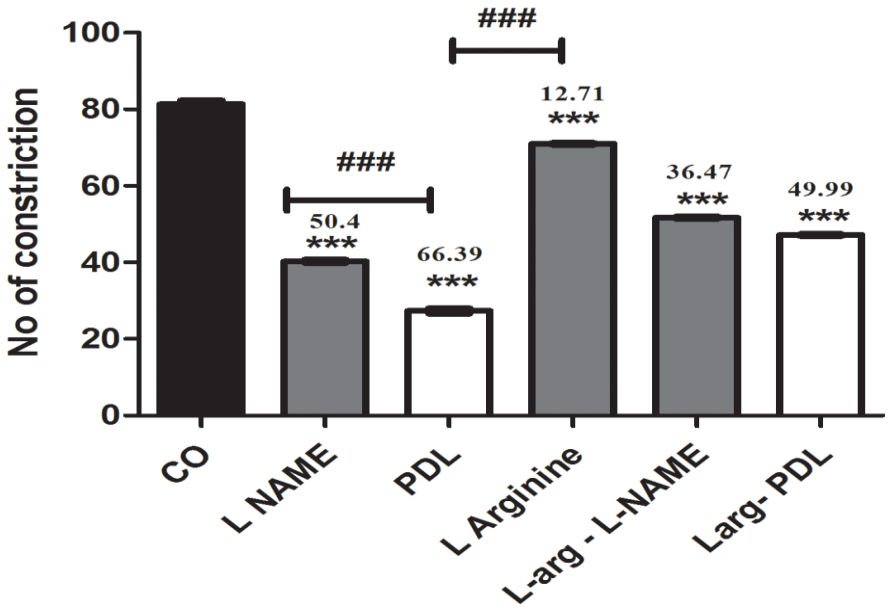

Fig. 7. Effects of antinociceptive properties of PDL (200 mg/kg) L- NAME (50mg/kg) pretreatment on L-arginine (50mg/kg) against Acetic acid- induced constriction model. CO: Control group. Values are mean ± SEM (n=6). The asterisks denote the significance levels comparing with the control group and ### represents L- arginine and L- NAME when compared to PDL (one-way ANOVA, followed by Dunnett's post hoc test): ***P<0.001 and $^{###}$P<0.001.

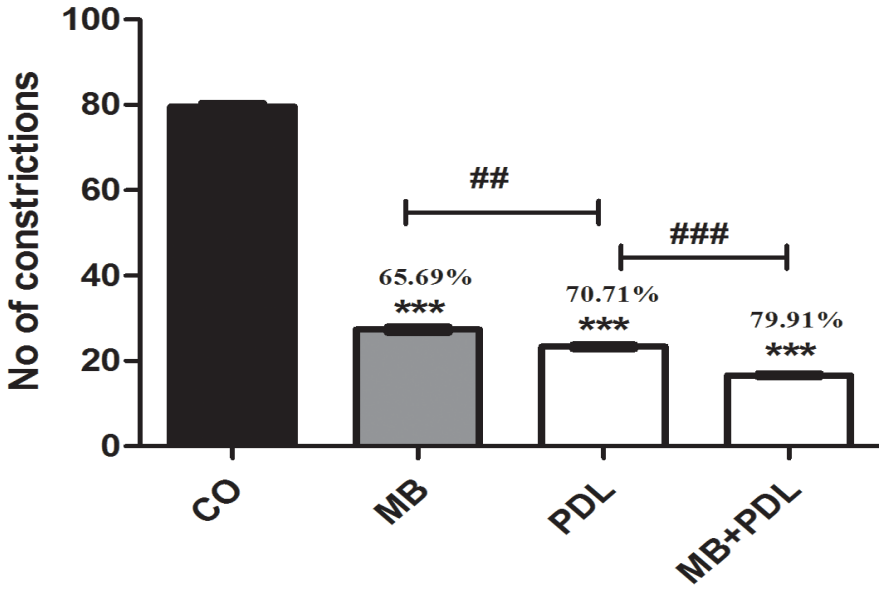

Fig. 8. Effects of Methylene blue (20mg/kg) pretreatment on PDL (200mg/kg) induced antinociceptive activity in acetic acid- induced abdominal writhing test in mice. Values are mean ± SEM (n=6). The asterisks * denote the significance levels comparing with the control group and ### represents MB when compared to PDL (one-way ANOVA, followed by Dunnett's post hoc test and Tukey's Multiple Comparison Test): ***P<0.001, $^{##}$P<0.01 and $^{###}$P<0.001.**

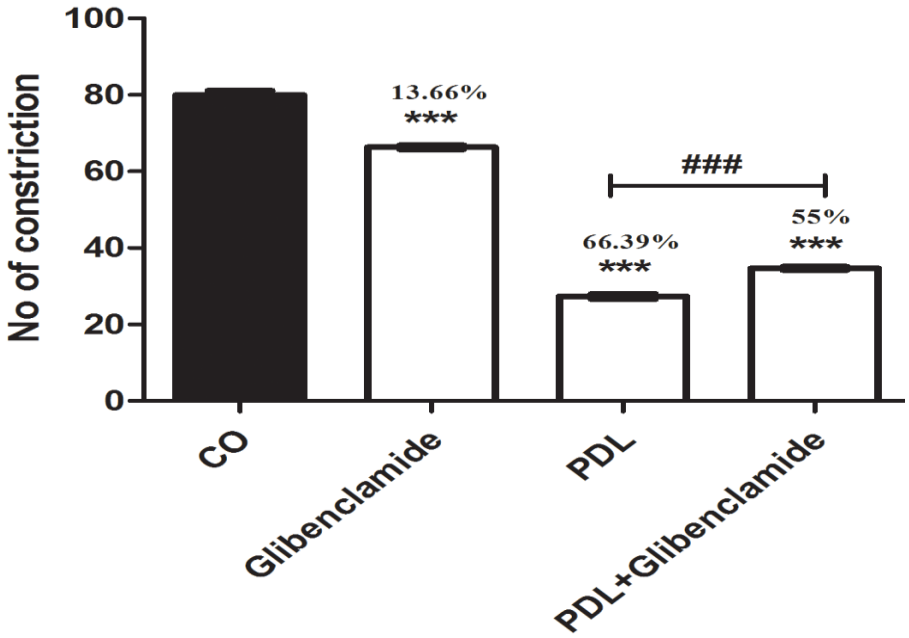

Fig. 9. Effects of glibenclamide (10 mg/kg) pretreatment on PDL (200mg/kg) induced antinociceptive activity in acetic acid induced writhing test. CO: Control group Values are mean ± SEM (n=6). The asterisks denote the significance levels comparing PDL with the control group and ### when compared to PDL treated group (one-way ANOVA, followed by Dunnett's post hoc test): ***P<0.001 and $^{###}$P<0.001.

MATERIALS AND METHODS

Animals

Healthy Male and female Swiss albino mice (25–30 g) and Wistar albino rats (250– 280 g) were used. Animals were housed under standard environmental conditions (temperature at 22 ± 2 ºC, humidity: 50 ± 10% and 12 h/12 h natural light/dark cycle) and fed with standard pellet diet and water ad libitum. Each treated groups consisted six animals per group. The experimental protocol of the study was approved by the Institutional Animal Ethics Committee (IAEC) of K.M. College of Pharmacy, Madurai, constituted under Committee for Purpose of Control and Supervision of Experiments on Animals (CPCSEA), approval no (IAEC/MKU/KMCP/41/2018).

Drugs and chemicals

N_ω-Nitro-L-arginine methyl ester hydrochloride (L-NAME), L-arginine hydrochloride (L- arginine), L-glutamic acid, capsaicin, methylene blue, glibenclamide, dimethyl sulfoxide (DMSO), acetylsalicylic acid (ASA) were purchased from Sigma-Aldrich Co. (Ind). All drugs were dissolved in physiological saline (0.9% NaCl) and buffered to pH 7, while PDL and ASA were dissolved in distilled water and DMSO respectively and the control received water. All drugs, chemicals were freshly prepared and administered subcutaneously or intraperitoneally (s.c. and i.p.) in volumes of 10 mL/kg unless otherwise stated in the method.

Acute Toxicity Study

According to OECD guidelines-425 [21], male Wistar rats were used for acute toxicity studies. Briefly, five groups of animals (n = 6) were used. First group was treated as standard control treated with distilled water. The groups (II-IV) were administered with PDL in doses of 30, 300, and 2000 mg/kg and group V belongs to toxic control. Animals were observed for 14 days. At the end of 14 days, the animals were sacrificed and histological studies performed on liver of the animals using haematoxylin – eosin (H&E) staining. Photomicrographs were taken with light microscope (Lab vision I-3000) at 40X.

Anti- Nociceptive analysis

Acetic acid-induced abdominal writhing test

The mice groups were divided into five groups of six each. The group I as control group and served with distilled water (10 mL/kg). Acetylsalicylic acid (ASA, 100 mg/kg, i.p) [22] as positive control group and dosed 30 min before the nociceptive agent. The group III-V were pre-treated with three different doses (50,100,200 mg/kg) of PDL. When the acetic acid injected intraperitoneally the animals were placed into a chamber and a number of constrictions was recorded for 30 min, starting from 5min post injection [23].

Formalin Test

Briefly, the PDL (50,100,200 mg/kg p.o.) was administered 30 min before the administration of ASA (100 mg/kg; as positive control), 20 μL of formaldehyde 2.5% (v/v) was injected subcutaneously into the plantar surface of the left hind paw of the rats. The behavioural responses to nociception including biting, licking and scratching of the injected

paw were noted [24]. The first 5 min were considered as the early phase and the period of 15-30 min as the late phase of the nociceptive response. To investigate the role of opioid system in the antinociceptive effect of PDL the rats were pretreated with naloxone (5 mg/kg, i.p.). After 15 min, the PDL (200 mg/kg, p.o.) was administered to the animals and the nociceptive response was measured 30 min thereafter.

Capsaicin-induced nociception test

The experimental mice were dosed with PDL (50, 100 and 200 mg/kg, p.o.), ASA (100 mg/kg, i.p.), capsazepine [25], a competitive antagonist for TRPV ion channels (0.17 mmol/kg, i.p.), or distilled water (10 mL/kg, p.o.) 30 min before intraplantar injection of 20 μL of capsaicin (1.6 g/paw) [26] into the right hind paw. Following capsaicin injection the mice were examined independently for 5 min. The time spent paw licking time of each mouse from its injected paw was noted as an indication of nociception.

Glutamate-induced nociception test

According to Beirith [27], 20 μL of glutamate (10 μmol/ paw) was injected into the ventral surface of the right hind paw of the mice. The mice were observed for once in 15 min following glutamate injection. The amount of time each mice spent for licking its injected paw was recorded as indicative of nociception. Mice were classified and dosed with PDL (50, 100 and 200 mg/kg, p.o.), ASA (100 mg/kg, i.p.) or distilled water (10 mL/kg, p.o.) 30 min before glutamate injection.

Analysis of the possible mechanism of action of PDL

Involvement of L-arginine/NO pathway

The mice were pretreated with L-arginine (100 mg/kg, s.c.), and after 15 min, the mice received PDL (200 mg/kg, p.o.), N^G-nitro-L-arginine methyl ester (25mg/kg, L-NAME, s.c.) (Abacioglu et al., 2000). Nociceptive responses against acetic acid injection were observed for 30 min. The numbers of abdominal writhing were counted as indication of pain behaviour.

Involvement of cyclic guanosine monophosphate (cG MP) pathway

To identify the feasible involvement of cGMP in the antinociceptive action caused by PDL, the experimental mice were pre-treated with methylene blue (a non specific inhibitor of NO, 10 mg/kg, s.c.) [28]15 min before the application of PDL (200 mg/kg, p.o.). The numbers of abdominal constriction were calculated as indications of pain behaviour were observed for 30 min after the acetic acid injection [29]

Involvement of ATP-sensitive K^+ channel pathway

The probable contribution of K^+ channel in the antinociceptive effect of PDL, the mice were administered with glibenclamide (an ATP-sensitive K^+ channel inhibitor, 10 mg/kg, i.p.) 15 min before the treatment of PDL (200 mg/kg, p.o.). The mice were served with 0.6% acetic acid i.p. 30 min post-treatment [30]. Subsequently the mice were immediately located in a Perspex chamber and the number of constrictions was registered for 30 min, starting from 5 min of post injection.

Statistical analysis

Results were expressed as mean ± SEM and analyzed by one-way ANOVA followed by Dunnett's post hoc and Tukey's multiple comparison test unless otherwise stated. $P < 0.01$and $P < 0.001$ was considered significant. The IC_{50} (dose that produced 50% inhibition in total time of paw licking) values were determined by using linear regression and graphs were drawn by using GraphPad Prism 5.0.

RESULTS

GC-MS analysis

A total of 10 major peaks were identified from PDL in GC-MS (Figure 1) with the major compounds constituted of 5-hydroxy furfural (55.7%), Heptasiloxane (49.6%), 2,4-Bis(1,1-dimethylethyl)-phenol (45.3%), 5-Isoxazolecarboxylic acid (42.0%), 9,12,15-Octadecatrienoic acid (23.8%), 9,12-Octadecadienoyl chloride (14.5%), 9,12,15-Octadecatrienoic acid (13.2%), [1,2-a]pyrazine-1,4-dione, hexahydro-3-(2-methylpropyl)- pyrrole (8.4%), 2,2-dihydroxy-1-phenyl- ethanone (8.06%).

Acute toxicity study (Histopathological -Liver)

The normal (Figure 2 1d control) liver showed polygonal hepatocytes with rounded nucleus, exhibiting normal central vein, and portal triads. In the toxic group (Figure 2 1e), remarkable change in cyto architecture was observed with lymphocyte infiltration, focal portal inflammation, distorted hepatic sinusoids. Group II, III and IV (30,300 and 2000mg/kg) (Figure 2 1a-c) showed normal histological features. Particularly, Group IV (Figure 2 1c) exhibited mild inflammation with dilated hepatic sinusoids, hepatocyte morphology remains intact and normal.

Acetic acid-induced abdominal writhing test

The results in Figure 3. described the efficacy of systemic administration of PDL (50, 100 and 200 mg/kg, p.o.) in acetic acid-induced abdominal writhing test. PDL exhibited significant dose-dependent inhibition on abdominal writhing. Interestingly PDL (200 mg/kg) showed better percentage of inhibition of 65.04% when compared to control ASA (100 mg/kg, i.p.) was 42.44%. The

deduced mean IC_{50} for p.o. administration of PDL was 169.9 mg/kg.

Formalin Test

PDL exhibited a significant ($p < 0.001$) antinociceptive activity in a dose-dependent manner in both phases of the formalin induced paw licking test as shown in Figure 4a and b, respectively. In comparison to PDL, morphine (5 mg/kg) also attenuated both phases while ASA (100 mg/kg) only reduced the nociception in the late phase. Overall, morphine was effective than the ASA and PDL in both phases of the formalin test. The IC_{50} values for PDL in both first and second phase were 136.6 and 174.8 mg/kg.

Capsaicin-induced nociception test

The results obtained from the Figure 5 demonstrated that application 50, 100 and 200 mg/kg of PDL via p.o. generated dose dependent neurogenic nociception showed percentage of inhibition 14.68, 32.93 and 56.74%, respectively. When compared to the control group, Only 50 mg/kg of PDL showed no difference while others displayed significant ($p < 0.001$) antinociceptive activity, moreover 200 mg/kg PDL showed better potential than with capsazepine (0.17 mmol/kg) (TRPV1 antagonist). The IC_{50} value for PDL in capsaicin-induced nociception was 194.9 mg/kg. The percentage of inhibition of ASA (33.73%) and capsazepine (50.79%) were used as positive control drugs exhibited somewhat identical inhibition when compared with PDL (56.74%) group.

Glutamate-induced nociception test

In (Figure 6) the glutamate induced nociception exposed that the administration of PDL (50, 100 and 200 mg/kg, p.o.) produced dose-dependent nociception with the percentage of inhibition observed of 12.23, 25.28 and 57.02%. The positive control drug ASA (100 mg/kg) was showed similar significant inhibition as compared to the control group at 51.07% somewhat lesser than PDL (200mg/kg). All treatment groups were showed significant antinociceptive activity when compared with control group other than 50mg/kg of PDL. The IC_{50} value for PDL in glutamate-induced nociception was 204.6 mg/kg.

Involvement of NO/cGMP Pathway

Figure 7 shows the participation of L-arginine/NO pathway was analyzed by pretreatment of mice with nitric oxide precursor L-arginine was failed to affect the acetic acid induced nociception but it was able to significantly reverse the antinociception of PDL (200 mg/kg) and L-NAME (25mg/kg). Figure 8 shows both PDL (200 mg/kg) and methylene blue (10 mg/kg) alone significantly inhibited acetic acid induced abdominal constriction test. While methylene blue significantly increased PDL induced antinociception compared to the treatment of PDL and methylene blue alone.

Involvement of ATP-sensitive K^+ channel pathway

The involvement of ATP sensitive K^+ channel pathway, in figure 9 summarises glibenclamide (10 mg/kg) administration alone did not change the abdominal writhing count when determined via 0.6% acetic acid i.p. When administered together, antinociceptive activity of PDL was remarkably inverted by glibenclamide.

DISCUSSION AND CONCLUSION

According to this study, the proper application of PDL at the doses of 50,100 and 200 mg/kg evoked remarkable dose dependent inhibition against chemical induced nociception test models. Besides, an important finding of this study was PDL displayed feasible involvement of the glutamatergic system, TRPV1 receptor and L-arginine / NO/ cGMP/ATP sensitive K^+ channel pathway in the PDL- induced antinociception in experimental animals. The findings exhibited that flavonoid rich PDL has an capability of free radical scavenging activity against DPPH and H_2O_2 (see supplementary) which is accompanying the presence of hydroxyl groups of a diverse group of plant polyphenolic compounds, such as flavonoids and phenolic acids. The total flavonoid content is found to be 16.55 ± 1.83 per mg QE/g. Besides, GC-MS and HPLC-DAD experiments effectively demonstrated the presence of Phenolic and flavonoid compounds in PDL (see supplementary). To the best of our knowledge, there has been no attempt Acetic acid induced abdominal constriction incites algesia by release of various proinflammatory endogenous mediators that instigate peripheral nociceptors [31]. The results from this study exhibited that PDL administered via p.o. produced significant dose dependent contraction in the number of acetic acid induced abdominal constrictions. Additionally the measured IC_{50} and percentage of inhibition are better than positive control Indomethacin. Capsaicin can activate C- or A∂-fibres which incites the release of neuropeptides, excitatory amino acids, nitric oxide, and a proinflammatory mediator which precisely activates non-selective ionotropic channel in primary sensory neurons [32]. In the present study, the probable involvement of TRPV1 receptor inhibition in the PDL induced antinociception was examined. PDL influences capsaicin induced nociceptive response in a dose dependent mode. Moreover the efficacy of capsaicin was antagonized with a TRPV1 receptor antagonist, capsazepin. These predictions specify that the antinociceptive activity of PDL probably involves the inhibition of TRPV1 receptors. Previously, the nociceptive response generated by glutamate involves peripheral, spinal and supraspinal sites of action with glutamate receptors (AMPA, kainate and NMDA receptors) plays a significant role modulating

this nociceptive response [33-34]. Interestingly, in this study one can find out that p.o. administration of PDL produced a dose dependent inhibition stimulated by i.p. ingestion of glutamate into the mice hind paw. This suggests that the antinociception induced by PDL is associated with interaction with any glutamate receptors in the glutamatergic system or meddling with the NO production. Hence, further studies are required to identify types of glutamate receptors that are involved in PDL induced antinociception. To determine the role of L-arginine/NO/cGMP pathway in the modulation of antinociceptive activity of PDL. In order to scrutinize the involvement of NO/cGMP pathway in the modulation of PDL antinociceptive efficacy, the extract was administered against L-arginine (as a NO donar), L-NAME (inhibitor of NOS) and MB (inhibitor of cGMP pathway). NO stimulates the activity of sGC and leading to formation of cGMP from guanosine triphosphate and also increase the level of cGMP which is crucial for functioning of nociceptors and finally calcium depletion happened [35]. Moreover, once the NMDA receptors were activated which increases intracellular calcium and the substrate L-arginine transformed into NO was catalyzed by NOS [28, 36]. The results clearly demonstrated that the pretreatment with L-arginine (100mg/kg) did not produce any changes to the acetic acid induced nociception but reversed PDL's antinociception indicating that the presence of reduced form of NO and failed to inhibit the PDL's antinociceptive potential as well as MB alone produce significant ($p < 0.001$) antinociceptive effect and enhanced the antinociceptive effect exercised by PDL. This suggests that MB encourages antinociception by successively inhibiting peripheral NOS and GC, resulting in NO interference [28]. NO has been interact directly or indirectly with various inhibitory and excitory neurotransmitters (GABA, NMDA, TRPV1 and Opioid) which are mediated in cGMP-dependent manner. Thus, it is suggesting that PDL acts via NO-mediated /cGMP dependent pathway [37].The contribution of ATP-sensitive K^+ channel inhibition in PDL induced antinociception demonstrated that glibenclamide antagonist (K^+ channel) remarkably reversed the antinociceptive activity induced by PDL. Notably, so many reports are available on glibenclamide particularly blocked ATP-sensitive K^+ channel and show no impact on other types of K^+ channel such as Ca^{2+} activated K^+ channel and voltage gated potassium channel [36, 38]. As a result, the present findings might suggest that PDL exercised its antinociceptive activity through the opening of ATP-sensitive K^+ channel that allows the efflux of K^+ ion, thus leading to membrane repolarization and/or hyperpolarization state which significantly reduces the membrane excitability [39]. Given together the present results contribute strong evidence on the participation of NO/cGMP/ATP-sensitive K^+ channel pathway in PDL induced antinociceptive activity.

CONCLUSION

In conclusion, PDL exerted a non-opioid antinociceptive activity at the peripheral and central levels via mechanisms involving modulation of the vanilloid receptors, glutamatergic system, and NO-mediated/cGMP dependent/ ATP sensitive K^+ channel pathway. Moreover, the flavonoid and phenol based bioactive compounds might be assigned to the antinociceptive efficacy of PDL. The antinociceptive activity of PDL might be attributed to the presence of flavonoid-based bioactive compounds, including quercetin. The ability to exert a non-opioid antinociceptive activity at the peripheral and central level suggests that the extract could be a good candidate for the development of new analgesic drug that is lack of dependence/ tolerance effects seen with morphine.

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